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Synthesis and Analytical Applications of a New Heterocyclic Bis-azo Dye: 2,6-bis(7-Hydroxyacenaphthyl-8-Azo)Pyridine

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A new heterocyclic bis-azo dye, 2,6-bis(7-hydroxyacenaphthyl-8-azo)pyridine (PBA) has been synthesized, which gives sensitive colour reactions with four metal ions. Reaction behaviour of this reagent with these metal ions, *viz.*, copper(II), zinc(II), cadmium(II) and mercury(II) has been established under various conditions. All of these metal ions form 1 : 2 complex (M : L) with the reagent. The molar absorptivities of the colour reactions are 4.2×10^4 L mole^{-1} cm^{-1} for copper (λ_{max} 526 nm), 5.0×10^4 L mol^{-1} cm^{-1} for zinc (λ_{max} 520 nm), 2.2×10^4 L mol^{-1} cm^{-1} for cadmium (λ_{max} 524 nm), 4.0×10^4 L mol^{-1} cm^{-1} for mercury (λ_{max} 520 nm). Other physico-chemical characteristics of the complexes are established spectrophotometrically. A number of foreign ions tested for their interference and the use of masking agents wherever necessary is tabulated. Copper(II) can be determined selectively with PBA and the recommended procedure has been applied to estimate copper levels in food and milk samples.

Key Words: Synthesis, Analytical, 2,6-Bis(7-hydroxyacenaphthyl-8-azo)pyridine.

INTRODUCTION

Heterocyclic azo dyes comprise the largest group of organic reagents usecl in the spectrophotometric determination of metal ions[1]. 1-(2-Pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)resorcinol (PAR) are the two important representatives of this class of reagents. We synthesized a new bis-azo dye, 2,6-bis(7-hydroxyacenaphthyl-8-azo)pyridine (PBA) using the Anderson and Nickless method[2, 3]. The synthesizecl azo dye showed a good sensitivity and high selectivity for copper(II) as well as a good selectivity for zinc(II), cadmium(II) and mercury(II) ions.

As is well known, copper is recognized as an essential micronutrient. In copper deficiency induced anaemia in spite of elevated levels of iron in liver, the rate of haemoglobin synthesis is significantly reduced. Recent studies also showed that the deficiency affects the cardiovascular system and causes extensive damage to heart and arteries[4, 5]. The Food and Nutrition Board[6] has proposed an allowance of *ca.* 2 mg of copper per day, for an adult, to prevent any symptom of deficit. As a part of a balanced diet, an adequate amount of copper is vital to ensure a wide range of health benefits. Therefore copper is an essential bio-element for human life. Though deficiency of this element is very rare in human beings but

its excess intake poses threat to human health. Keeping in view the biological importance of copper it was thought worthwhile to determine the copper levels in some foodstuffs which are usually consumed by the local gentry of this area using the present reagent.

EXPERIMENTAL

Synthesis of 2,6-bis(7-hydroxyacenaphthyl-8-azo)pyridine (PBA): 0.01 Mol of 2,6-hydrazinopyridine was dissolved in minimum amount of glacial acetic acid. Separately, 0.02 mol of acenaphthoquinone was dissolved in ethanol. The two solutions were then mixed and left overnight. The mixture solution was neutralized with aqueous ammonia. The yellow coloured solid so obtained was filtered. The compound was recrystalized from ethanol. The purity of the compound was checked by thin layer chromatography. The coupling resulted in an azo dye as confirmed by infra-red spectrum with the complete absence of ν(C=O) frequencies at 1790 and 1720 cm^{-1}, but had a strong peak at 3300 cm^{-1}, showing thereby the enol form of the compound.

A 5×10^4 M solution of PBA was prepared by dissolving 0.2335 g in 1 L of ethanol.

Metal ion solutions: 0.001 M stock solutions of copper(II), zinc(II), cadmium(II) and mercury(II) were prepared by dissolving appropriate amounts of analytical grade copper sulfate pentahydrate, zinc sulfate heptahydrate, cadmium acetate dihydrate and mercuric chloride respectively, in doubly distilled water. Solutions were standardized complexometrically with EDTA and diluted further as required for working standards.

Buffer solutions[7]**:** *Phosphate buffer, pH 8.0:* This buffer was prepared by mixing 50 mL of 0.2 M potassium dihydrogen phosphate with 46.9 mL of 0.2 M sodium hydroxide solution and diluting to 200 mL with distilled water.

Borate buffer, pH 10: This buffer was prepared by diluting 250 mL of a solution containing 12.3690 g of boric acid and 24.9110 g of potassium chloride per litre and 220 mL of 0.2 M sodium hydroxide solution to 1 L with distilled water.

Foreign ion solutions: Solutions of various ions of suitable concentration were prepared using analytical grade reagents. Metal ion solutions were prepared from their acetate or sulfate salts and anions from their sodium or potassium salts.

All the reagents used were of analytical grade and doubly distilled water was used throughout.

A Bausch and Lomb Spectronics 2000 spectrophotometer with 10 mm matched glass cells was used for absorbance measurements.

Beckman pH-meter model Φ60 and a digital pH-meter Nig 333 model with glass electrodes of 0–14 pH ranges were used for pH adjustments.

Procedures

For copper(II): To an aliquot containing 3.0 to 19.0 μg of copper(II) ions, add 5 mL of 5×10^{-4} M reagent solution, 2 mL of phosphate buffer (pH 8), 1 mL of 1% potassium iodide solution and 10 mL of water.

For zinc(II): To an aliquot containing 3.2 to 13.0 μg of zinc(II) ions, add 6 mL of 5×10^{-4} M reagent solution, 2 mL of borate buffer (pH 10), 1 mL of 2.5×10^{-3} M EDTA and 10 mL of water.

For cadmium(II): To an aliquot containing 8.0 to 40.0 μg of cadmium(II) ions, add 4 mL of 5×10^{-4} M reagent solution, 2 mL of borate buffer (pH 10) and 10 mL of water.

For mercury(II): To an aliquot containing 15.0 to 50.0 μg of mercury(II) ions, add 5 mL of 5×10^{-4} M reagent solution, 2 mL of borate buffer (pH 10) and 10 mL of water.

Extract the complex in 10 mL of chloroform and record the absorbance at the respective λ_{max} against a corresponding reagent blank prepared under similar conditions.

RESULTS AND DISCUSSION

PBA is insoluble in water, slightly soluble in ethanol and methanol and highly soluble in water-immiscible solvents like chloroform, carbon tetrachloride, benzene, etc. It shows a slight yellow colour on protonation in highly acidic media and intense yellow in neutral and deep red color in highly alkaline media due to ionization.

Ethanolic solution of PBA gives colour reactions only with four metal ions, *i.e.*, with very dilute solutions of copper, zinc, cadmium and mercury in a slightly alkaline medium. The complexes are precipitated if the ethanolic concentration is kept below 50% (v/v). However, the precipitates are extractable in water-immiscible solvents, but colour fading continues when complexed with copper. Further investigations revealed that the colour of the copper complex can be stabilized for 2–3 h, if 1% potassium iodide solution is used as a stabilizer. The colours of the complexes retained in chloroform after extraction from alkaline solutions are as follows: copper(II) gives a dark red, zinc(II) a violet, cadmiium(II) a red and mercury(II) a blue coloured complex with PBA in neutral to slightly alkaline media. Studies also revealed that zinc, cadmium and mercury do not show any colour reaction if phosphate buffers are used while detennining copper, as phosphate seriously interferes in these cases. Thus copper can be selectively determined in the presence of other metals. Borate buffer, pH 10, is found to be the best suited for zinc, cadmium and mercury systems to maintain their maximum and constant absorbances.

Various physico-chemical and analytical characteristics of the complexes are summarized in Table-1.

Effect of diverse ions: In the determination of copper(II), zinc(II), cadmium(II) and mercury(II) at the level of 1.27 μg/mL, 1.3 μg/mL, 2.25 μg/mL and 4.0 μg/mL respectively, fluoride, chloride, bromide, nitrate, acetate, sulfate, sulfide, sulfite, tartarate, borate (up to 1000-fold), calcium, strontium, barium, titanium, vanadium, niobium, tantalum, aluminium, lanthanides (up to 100-fold) did not interfere.

The tolerance of other anions and cations which did not cause a deviation of ±2% in absorbance and which interfered seriously, in addition to masking possibilities, are listed in Table-2.

TABLE- 1
PHYSICO-CHEMICAL AND ANALYTICAL CHARACTERISTICS OF METAL ION COMPLEXES

Characteristics	Cu(II)-PBA Complex	Zn(II)-PBA Complex	Cd(II)-PBA Complex	Hg(II)-PBA Complex
λ_{max}, nm	526	520	524	520
pH range	7.8–10.0	9.5–10.5	9.0–11.0	8.5–10.5
Reagent required for maximum complexation/mole of metal ion	10	10	5	5
Beer's law validity range (ppm)	0.0–2.5	0.0–1.85	0.0–4.5	0.0–7.0
Optimum concentration range (ppm)	0.31–1.90	0.32–1.30	0.8 –4.0	1.5–5.0
Sandell's sensitivity ($\mu g/cm^2$)	0.0015	0.0013	0.005	0.005
Molar absorptivity	4.3×10^{-4}	5.0×10^{-4}	2.2×10^{-4}	4.0×10^{-4}
Composition (M : L) by Job's method	1 : 2	1 : 2	1 : 2	1 : 2

TABLE-2
EFFECT OF DIVERSE IONS

Foreign ions	Copper(II)	Zinc(II)	Cadmium(II)	Mercury(II)
EDTA	SI	150	40	40
Iodide	NI	NI	100	SI
Thiourea	NI	NI	100	SI
Thiosemicarbazide	SI	NI	500	SI
Oxalate	100	NI	NI	NI
Thiocyanate	80	NI	NI	NI
Cyanide	NI	NI	400	NI
Phosphate	NI	SI	SI	SI
Silver(I)	5	6	10	6
Manganese(II)	30	30	30	25
Iron(II)	10	10	8	15
Cobalt(II)	10	8	22	20
Nickel(II)	5	12	25	15
Copper(II)	—	10[a]	6[a]	SI
Zinc(II)*	5	—	SI	SI
Cadmium(II)*	5	SI	—	SI
Mercury(II)*	10	8[a]	5[a]	—
Palladium(II)	8	10	10	10
Lead(II)	35	25	50	55
Chromium(II)	40	35	40	50
Gold(III)	10	12	12	8

SI serious interference; * no color reaction in phosphate buffer;
NI no interference (1000-fold) [a] masked by thiosemicarbazide

Determination of copper in food and milk samples[7, 8]

Milk samples: 100 mL of milk (procured from local dairies) was added dropwise to a heated crucible to evaporate it without frothing. After the moisture had been removed, it was heated strongly to 450–500°C for 1 h. Utmost care was taken to avoid loss by sputtering. The white ash obtained was dissolved in minimum volume of diluted nitric acid and the volume was made up to 25 mL.

Food samples: 5 g foodgrains (dried for *ca.* 24 h at 70°C in an oven) were wet-ashed with nitric and perchloric acids. 5 mL of hydrochloric acid (1 + 9) was added to the ash and evaporated to dryness. This step was repeated. The dry residue was dissolved in water and filtered into a 25 mL standard flask, with the addition of 1 or 2 clrops of concentrated hydrochloric acid and made up to 25 mL.

The copper levels estimated in various samples are given in Table-3.

TABLE-3
CONTENTS OF COPPER IN VARIOUS FOOD AND MILK SAMPLES

Sample	No. of analysis	Sample ashed (mL or g)	Cu found in whole sample (μg)	Cu found in whole sample using AAS (μg)	Range of Cu levels (mg/100 mL or mg/100g)
(a) Food Grains					
Triticum aestivum (Wheat)	3	5	16.52, 16.95, 16.00	16.36, 16.80, 16.20	0.320–0.340
Oryza sativa (bran-rice)	5	2	10.10, 10.24, 10.49, 11.88, 9.97	10.40, 10.82, 10.42, 10.82, 10.13	0.497–0.594
Zea mays (Maize)	4	4	54.55, 55.08, 55.89, 56.81	54.98, 55.00, 55.60, 55.90	1.377–1.420
Phaseolus aureus (Mung)	4	2	8.66, 9.58, 8.32, 9.51	8.60, 8.94, 8.20, 9.20	0.416–0.479
Lens culinaris (Masur)	4	2	10.60, 13.00, 10.60, 11.20	12.40, 12.98, 12.60, 13.20	0.530–0.650
Pisum sativum (Peas)	4	4	13.20, 16.40, 17.20, 20.40	13.68, 16.98, 17.50, 19.20	0.330– 0.510
Arachis hypogaea (Peanuts)	4	4	21.20, 22.40, 27.60, 24.00	22.88, 25.24 25.68, 24.10	0.530–0.690
(b) Milk Samples					
Cow milk	4	100	22.90, 23.40, 21.70, 25.20	25.40, 22.80, 22.10, 25.50	0.022–0.025
Buffalo milk	4	100	25.50, 24.80, 29.90, 23.00	30.10, 24.10, 29.20, 22.80	0.023–0.030
Goat milk	4	100	38.80, 44.00, 41.50, 37.50	39.00, 43.40, 40.90, 37.50	0.039–0.044
Milk powder	4	5	15.45, 15.50, 14.40, 16.40	15.50, 15.20, 15.00, 16.30	0.288–0.328

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